Appendix C. Ecological Toxicity Assessment

Ecological Toxicity Summary

On an acute basis, molinate is practically nontoxic to birds and slightly toxic to mammals. Molinate is a reproductive toxicant in mammals, with a chronic NOAEL for ecologically relevant effects at 5 ppm. The chronic toxicity to birds is unknown. Most studies indicate that the molinate is slightly toxic to freshwater and saltwater fish on an acute basis, although a couple of studies indicate that it is highly toxic. Carp are particularly sensitive to subchronic exposure of molinate, with LC_{50}'s from 2-3 exposure studies of approximately 0.2 ppm. The reported chronic NOAEL for fish is 390 ppb, but this value may underestimate the toxicity of molinate because it came from a test which did not assess reproductive effects. Molinate is moderately to highly toxic to both freshwater and saltwater crustaceans on an acute basis. The chronic NOAEL is 110-380 ppb for freshwater crustaceans and 25.6 ppb for saltwater crustaceans. Molinate is slightly toxic to estuarine mollusks. For an herbicide, molinate is not particularly toxic to nontarget plants. Based on vegetative vigor effects, molinate may harm terrestrial plants at an exposure of 0.22 lb/A or more. The EC_{50}'s for sensitive aquatic plants are 3.3 ppm for vascular plants and 0.22 ppm for algae. All toxicity values are given in terms of active ingredient.

a. Toxicity to Terrestrial Animals

i. Birds, Acute and Subacute

An acute oral toxicity study using the technical grade of the active ingredient (TGAI) is required to establish the toxicity of molinate to birds. The preferred test species is either mallard duck (a waterfowl) or bobwhite quail (an upland game bird). Results of this test are shown below.

Acute Oral Toxicity to Birds

Species	% ai	LD_{50} (mg/kg)	Toxicity Category	MRID No. Author/Year	Study Classification[a]
Mallard *(Anas platyrhynchos)*	98.8	>2250[b]	Practically nontoxic	Acc. # 258924 Beavers, 1984	Core

[a] Core studies satisfy test guideline requirements; supplemental studies are scientifically sound, but do not satisfy guideline requirements.
[b] Neither mortality nor adverse effects were observed at any test level.

Since the LD_{50} is greater than 2000 mg/kg, molinate is classified as practically nontoxic to avian species on an acute oral basis. The guideline (71-1) is fulfilled (Acc. No. 258924).

Two subacute dietary studies using the TGAI are required to establish the toxicity of molinate to birds. The preferred test species are mallard duck and bobwhite quail. Results of these tests are tabulated below.

Subacute Dietary Toxicity to Birds

Species	% ai	5-Day LC50 (ppm)[a]	Toxicity Category	MRID No. Author/Year	Study Classification
Northern bobwhite quail *(Colinus virginianus)*	98.8	> 5620[b]	Practically nontoxic	Acc. # 258924 Beavers, 1984	Core
Mallard duck *(Anas platyrhynchos)*	98.8	>5620[c]	Practically nontoxic	Acc. # 258924 Beavers, 1984	Core
Mallard duck *(Anas platyrhynchos)*	72.3 (Ordram 6E)	21,100 (15,300 ppm ai)	Practically nontoxic	Acc. # 246020 Beliles *et al*., 1965	Supplemental[d]

[a] Test organisms observed an additional three days while on untreated feed.
[b] Mortality at 1780 ppm and 3160 ppm were 10% and 30%, but none of these mortalities was attributed to the test substance.
[c] No mortality was observed at any of the test levels.
[d] Study was supplemental because a formulated product was tested.

Since the LC_{50}'s are greater than 5000 ppm, molinate is classified as practically nontoxic to avian species on a subacute dietary basis. The guideline (71-2) is fulfilled (Acc. No. 258924).

ii. Birds, Chronic

Avian reproduction studies using the TGAI are required for all pesticides with outdoor uses. Data on the effects of molinate on the reproduction of birds was previously listed as "reserved", but is now required. These data are especially important since mammalian studies indicate that molinate can cause reproductive toxicity. Avian reproduction studies with the mallard and the bobwhite are required. The guideline (71-4) is not fulfilled.

iii. Mammals, Acute and Chronic

For molinate, the rat toxicity data obtained from the Agency's Health Effects Division (HED) will substitute for wild mammal testing. These toxicity values are reported below.

Acute Toxicity to Mammals

Species	% ai	LD_{50} (mg/kg)	Toxicity Category	MRID/Acc. No.
Rat (*Rattus norvegicus*)	96.0	549	Slightly toxic	247547
Rat (*Rattus norvegicus*)	99.5	*male:* 584 *female:* 660	Slightly toxic	
Rat (*Rattus norvegicus*)	69.3 (Ordram)	940 (651 mg ai/kg)	Slightly toxic	250520
Rat (*Rattus norvegicus*)	69.3 (Ordram)	852 (590 mg ai/kg)	Slightly toxic	250520
Rat (*Rattus norvegicus*)	71 (Ordram 6-E)	*male:* 794 (534 mg ai/kg) *female:* 681 (484 mg ai/kg)	Slightly toxic	
Rat (*Rattus norvegicus*)	10 (Ordram)	>5000 (>500 mg ai/kg)	Practically nontoxic	249409

The results indicate that molinate is slightly toxic to small mammals on an acute oral basis. Results from chronic and subchronic rat studies pertinent to ecological effects are tabulated below.

Subchronic and Chronic Toxicity to Mammals

Species	% ai	Test Type	Toxicity Value	Affected Endpoints	MRID/Acc. No.
Rat (*Rattus norvegicus*)	99.5	13-week feeding	NOEL=700 ppm LOEL=1400 ppm	Decreased organ weight, histological changes	002264
Rat (*Rattus norvegicus*)	99.5	13-week feeding	NOEL=160 ppm LOEL=320 ppm	Ovarian vacuolation	002264
Dog	99.5	13-week feeding	NOEL=900 ppm LOEL=1800 ppm	Increased thyroid weight	002264
Rat (male)		Fertility study, 5 days, 5 weeks, and 10 weeks	NOEL=0.2 mg/kg/day (4 ppm) LOEL=4 mg/kg/day (80 ppm)	Decreased number, viability and mobility of sperm, increased sperm abnormalities, decreased number of implants and fetuses, increased pre-implantation loss.	Acc. No. 245675
Rat	Technical	3-month inhalation	NOEL not determined LOEL=2.2 mg/m^3	Testicular degeneration, abnormal spermatozoa	Acc. No. 241965
Rat	Technical	3-month inhalation	NOEL not determined LOEL=2.2 mg/m^3	Decreased number of implantations and fetuses	Acc. No. 241965
Rat	86.6	90-day neurotoxicity	NOEL not determined LOEL=50 ppm	Body weight, food consumption and utilization	MRID 43270701
Mouse	97.6	18=month carcinogenicity	NOEL=10 ppm LOEL=100 ppm	Testicular degeneration	MRID 41809201, 43037801
Rat		Developmental toxicity	NOEL=44 ppm LOEL=700 ppm	Increase in runting	MRID 41473401
Rat		Developmental toxicity	NOEL=400 ppm LOEL=4000 ppm	Increased abortions, decreased maternal weight gain, increased maternal liver wt, delayed fetal development	MRID 14021015
Rabbit	98.8	Developmental toxicity	NOEL=20 mg/kg/d (~660 ppm) LOEL=200 mg/kg/d (~6600 ppm)	Increased abortions, decreased maternal body weight gain,increase liver weight, reduced ossification of the sternbrae.	MRID 001474-30
Rabbit	98.1	Male fertility	NOEL=40 mg/kg/d (~1320 ppm) LOEL=80 mg/kg/d (~2640 ppm)	Atypically sperm	MRID 425613-01
Rat	97.6	2-generation reproduction	*Maternal:* NOEL=6 ppm LOEL=50 ppm *Repro:* NOEL=6 ppm LOEL=50 ppm	Vacuolation/hypertrophy of the ovary Decreased fecundity, vocuolation/hypertrophy of ovary, ovarian lesions in offspring	MRID 41333402
Rat		2-generation reproduction	*Parental:* NOEL=5 ppm LOEL=10 ppm *Repro:* NOEL=5 ppm♂/ 20 ppm♀	Increased sperm abnormalities Lesions in ovary, increased sperm abnormalities,	MRID 44403201

Subchronic and Chronic Toxicity to Mammals

Species	% ai	Test Type	Toxicity Value	Affected Endpoints	MRID/Acc. No.
Rat	Technical	3-generation reproduction	NOEL>12.6 ppm	--	231331
Rat	Technical	2-year feeding/ carcinogenicity	NOEL not determined LOEL<12.6 ppm	Increased organ and testes weight	231327 and 236576
Rat	97.6	2-year feeding/ carcinogenicity	*Reproductive:* NOEL=7 ppm LOEL=40 ppm	Ovarian lesions, degeneration with atrophy of testes and decreased testes weight at 300 ppm.	MRID 41815101, 43037801, and 43116302
			Neurotoxicity: NOEL < 7 ppm LOEL = 7 ppm	Degeneration/demethylation in the sciatic nerve and atrophy/reserve cell hyperplasia in the muscle.	
Rat	96.8	Neurotoxicity	*Mat:* NOEL=300 ppm LOEL=700ppm	Decreased body weight gain and food consumption	MRID 4338202
			Repro: NOEL=700ppm LOEL=1000ppm	Increased gestation length, decreased litter size, decreased percent of male pups, decrease number of pups born alive, and death of all pups by day 4 post partum.	
Rat		22-day *post partum partum partum*	NOEL not determined LOEL=300 ppm	Delayed vaginal opening	MRID 44373601

Molinate shows a clear pattern of causing reproductive toxicity in rodents. Numerous studies involving different routes of exposure (dietary, inhalation, and injection) show that molinate exposure damages testes and ovaries and reduces fertility in males and females. Studies also show that sexual development in offspring is impaired. Although the mechanism of action is uncertain, these results are consistent with molinate possibly being an endocrine disruptor. In addition to reproductive and developmental effects, molinate has been shown to be a neurotoxin Neurotoxic effects have been observed in offspring following *in utero* exposure at dose levels at or below the maternal NOAEL. Studies with rabbits and dogs suggest that nonrodent species are considerably less sensitive to the reproductive toxicity of molinate than are rodents.

For the purpose of the risk assessment for wild mammals, the two-generational study with the rat (MRID 4440320) established the NOAEL as 5 ppm and the LOAEL as 10 ppm for male mammals. For female mammals, a second 2-generational study with the rat (MRID 41333402) established the NOAEL and LOAEL as 6 ppm and 50 ppm, respectively. These NOAEL and LOAEL values are based on endpoints that are known to be ecologically significant, including reduced number and survival of offspring (i.e., fertility).

Published literature provides further evidence of the reproductive toxicity of molinate. Jewell *et al.* (1998) found that *ip* administration of molinate at doses of 200 to 400 mg/kg produced tissue

damage in the testes of male rats. The testes in the rats of the 400 mg/kg showed severe atrophy, weighing less than half of the testes of control rats, and were almost completely absent of germ cells. Although this study did not look at reproduction, the results indicate that these rats became essentially infertile. Similar effects were observed in rats that were administered molinate sulfoxide, a primary metabolite of molinate, suggesting that the toxic moiety is molinate sulfoxide or another metabolite that is produced further down the metabolic pathway. The authors concluded that reproductive toxicity of the sulfoxide metabolite exceeds that of the parent.

Ellis et al. (1998) found similar effects on reproduction, but at lower doses, when they administered molinate and molinate sulfaxide to male rats for a duration of 7 days. When administered at 40 mg/kg/day (approximately 800 ppm) molinate produced a sperm lesion and caused markedly decreased concentrations of circulating and testicular testosterone. Morphological changes in the testes were observed at higher doses (140 mg/kg/day). Administration of the metabolite molinate sulfoxide at 10 mg/kg/day (approximately 200 ppm) or more produced a similar sperm lesion and caused markedly decreased plasma and testicular concentrations. The results provide further evidence that metabolic activation of molinate, which involves the oxidation of the molinate sulfur, results in testis damage and reproductive impairment.

iv. Insects

A honey bee acute contact study using is not required for molinate because its use only on rice, a crop which is not associated with much honey bee exposure. Therefore, testing of molinate for nontarget insect toxicity is not required.

b. Toxicity to Freshwater Animals

i. Freshwater Fish and Amphibians, Acute and Subchronic

Two freshwater fish toxicity studies using the TGAI are required to establish the toxicity of molinate to fish. The preferred test species are rainbow trout (a coldwater fish) and bluegill sunfish (a warmwater fish). Results of these tests are tabulated below.

Acute Toxicity of Technical Molinate to Freshwater Fish

Species/ (Flow-through or Static)	% ai	96-hour LC50 (ppm)	Toxicity Category	MRID/Acc. No. Author/Year	Study Classification
Rainbow trout *(Oncorhynchus mykiss)* flow-through	96.8	20 (17-27)	Slightly toxic	MRID 43337603 Kent *et al.*, 1994	Core
Rainbow trout *(Oncorhynchus mykiss)* static	98.6	0.21 (0.16-0.29)	Highly toxic	MRID 40098001 Mayer and Ellersieck, 1986	Core
Rainbow trout *(Oncorhynchus mykiss)* static	99	6.97 (5.21-9.34)	Moderately toxic	Acc. No. 246020 Sleight *et al.*, 1970	Supplementary
Rainbow trout *(Oncorhynchus mykiss)* static	97.8	1.3 (0.896-1.88)	Moderately toxic	Acc. No. 246020 Beliles *et al.*, 1983	Supplementary
Bluegill sunfish *(Lepomis macrochirus)* flow-through	96.8	23.1 (18-32)	Slightly toxic	MRID 43337602 Kent *et al.*, 1994	Core
Bluegill sunfish *(Lepomis macrochirus)* static	98.6	0.32 (0.19-0.53)	Highly toxic	MRID 40098001 Mayer and Ellersieck, 1986	Core
Bluegill sunfish *(Lepomis macrochirus)* static	99	18.8 (16.7-21.1)	Slightly toxic	Acc. No. 246020 Sleight *et al.*, 1970	Supplementary
Bluegill sunfish *(Lepomis macrochirus)* static	97.8	29 (20.4-39.7)	Slightly toxic	Acc. No. 246020 Beliles *et al.*, 1983	Supplementary
Catfish (unknown species)	Technical	13.0 (10.6-16.0)	Slightly toxic	Acc. No. 246020 McGowan, 1972	Supplementary
Fathead minnow (*Pimephales promelas*) static	99	26.0 (20.5-32.9)	Slightly toxic	Acc. No. 246020 Sleight *et al.*, 1970	Supplementary
Carp (*Cyprinus carpio*) static	Technical	42.8 (32-56)	Slightly toxic	Acc. No. 246020	Supplementary
Goldfish (*Carrassius auratus*)	97.8	30 (16.2-55.5)	Slightly toxic	Acc. No. 246020 Beliles *et al.*, 1983	Supplementary

Below are toxicity results for formulated products of molinate.

Acute Toxicity of Formulated Products of Molinate to Freshwater Fish

Species/ (Flow-through or Static)	% ai	96-hour LC50 (ppm)	Toxicity Category	MRID/Acc. No. Author/Year	Study Classification
Rainbow trout *(Oncorhynchus mykiss)* static	90.3 (Ordram 8E)	19.5 (9.8-31) (17.6 mg ai/L)	Slightly toxic	MRID 41613603 Tapp *et al*., 1990	Core
Rainbow trout, stealhead form *(Oncorhynchus mykiss*), static	90.3 (Ordram 8E)	14 (4.7-23.3)	Slightly toxic	Finlayson and Faggella, 1986	Unreviewed (Open literature)
Chinook salmon (*Oncorhynchus tshawytscha*)	90.3 (Ordram 8E)	13 (10.8-15.2)	Slightly toxic	Finlayson and Faggella, 1986	Unreviewed (Open literature)
Bluegill sunfish *(Lepomis macrochirus)* static	90.3 (Ordram 8E)	24 (18-33) (21.7 mg ai/L)	Slightly toxic	MRID 41613601 Sankey *et el*., 1990	Core
Channel catfish (I*ctalurus punctatus*)	90.3 (Ordram 8E)	34 (20-48)	Slightly toxic	Finlayson and Faggella, 1986	Unreviewed (Open literature)
Striped bass (*Morone saxatilis*)	90.3 (Ordram 8E)	8.1 (6.4-9.8)	Slightly toxic	Finlayson and Faggella, 1986	Unreviewed (Open literature)
Mosquito fish (*Gambusia affinis*)	71.0 (Ordram 6E)	26 (18 mg ai/L)	Slightly toxic	MRID 00084743 Bullock, 1968	Supplementary

The acute toxicity of molinate to fish is uncertain because of the unusually wide range of reported toxicity values. The majority of the studies place the toxicity of molinate in the "slightly toxic" category (>10 to 100 ppm); however, two supplemental studies place it in the "moderately toxic" category (>1 to 10 ppm), and two studies reported in the EPA reference manual of Mayer and Ellersieck (1986) place it in the "highly toxic" range (0.1 to 1 ppm). These data indicate that the rainbow trout or the striped bass is the most sensitive species tested for acute toxicity, although under subchronic exposure the common carp might be even more sensitive (see below). Because of the large discrepancy in reported toxicity values, both the minimum value for the rainbow trout (0.21 mg ai/L) and the mean of the six LC_{50} values from studies with rainbow trout (10.3 mg ai/L) will be used in the risk assessment for freshwater fish. These data fulfill the requirements of guideline 72-1 (MRIDs 43337602, 43337603, 41613601, 41613603, 40098001, Acc. No. 246020).

Other studies with a formulated product have shown that subchronic exposure (21-28 days) to molinate can cause mortality in fish at concentrations lower than those that are toxic at acute exposure. The mechanism of the subchronic mortality appears to be induced anemia (Kawatsu, 1977). Finlayson and Faggella (1986) concluded that the mortality of an estimated 7,000 to 30,000 common carp in the Colusa Basin Drain in California from 1981 through 1983 could be attributed to high molinate concentrations resulting from drainage of water from rice fields. The following table summarized finding of subchronic toxicity of fish by molinate.

Subchronic Toxicity to Freshwater Fish

Species/ (Flow-through or Static)	% ai	Subchronic LC50 (ppm)	NOAEL (ppm ai)	LOAEL (ppm)	Endpoint effected	MRID/Acc. No. Author/Year
Common carp (*Cyprinus carpio*)	90.3 (Ordram 8-EC)	0.21 (28-day)	0.09	0.13	Survival, hematocrit and hemoglobin levels	Finlayson and Faggella, 1986
Common carp (*Cyprinus carpio*)		0.18 (21-day)	0.032			Kawatsu, 1977
Channel catfish (*Ictalurus punctatus*)	90.9 (Ordram 8E)	6.1 (4.5-8.1)	0.88	1.57	Behavior, hematocrit and hemoglobin levels	Miller 1984 Acc. No. 258924
Channel catfish (*Ictalurus punctatus*)	90.3 (Ordram 8-EC)	>8.6	1.7	2.6	Hematocrit and hemoglobin levels	Finlayson and Faggella, 1986
Bluegill sunfish *(Lepomis macrochirus)*	90.9 (Ordram 8E)	>6.05	6.05	--[2]	None	Miller 1984 Acc. No. 258924

[1] These toxicity categories were established for acute toxicity.
[2] No adverse effects were observed at any test concentration.

These results show that exposure to molinate concentrations as small as 0.13 ppm for 28 days can cause significant declines in hematocrit and hemoglobin levels, signifying anemia, which can lead to mortality of fish. The study with the channel catfish found that hematocrit and hemoglobin levels returned to normal by day 42 of the recovery period.

Heath *et al*. (1997) studied mortality and sublethal effects in the fathead minnow in response to acute exposure to molinate. The results from this study are somewhat unreliable because a problem with low dissolved oxygen compromised the test of molinate toxicity. Increased mortality and slowed growth were observed in fish exposed to 83 μg/L and 9700 μg/L of molinate, but these responses were not dose-related and could have been caused by the low DO conditions. A reduction in swimming response was observed in fish exposed to a concentration near the LC_{50} (9700 μg/L), but not in fish exposed to a concentration that approximated molinate levels in the Colusa drain (83 μg/L). Neither concentration level resulted in observable effects on acetylcholinesterase activity or critical thermal minimum and maximum levels. Finally, in two trials testing fish water collected from the Colusa Basin Drain, mortality was at low background levels and fish showed no adverse sublethal effects. The water samples were taken from the Colusa Basin Drain on May 21 and June 6, 1994, a time of the year of peak use of molinate on rice. This paper concluded that levels of molinate that currently occur in Colusa Basin Drain (after the imposition of mandatory water-holding periods on rice fields) cause no acute or measurable sublethal effects in fish.

The table below gives results of tests of the toxicity Ordram:Propanil 3:3E, a formulated product containing propanil as well as molinate. The percent active ingredients of this product were not specified.

Acute Toxicity of Ordram:Propanil 3:3E to Freshwater Fish

Species/ (Flow-through or Static)	96-hour LC50 (ppm)	Toxicity Category	MRID No. Author/Year	Study Classification
Rainbow trout *(Oncorhynchus mykiss)* static	8.3 (5.6-18)	Moderately toxic	41613604	Core
Bluegill sunfish *(Lepomis macrochirus)*	14 (11-18)	Slightly toxic	41613602	Core

These results indicate that Ordram:Propanil 3:3E is slightly to moderately toxic to freshwater fish on an acute basis.

One study is available on the toxicity of molinate to amphibians. Sanders (1970, Acc. No. 246020, Reference No. 25) tested the acute toxicity of molinate to tadpoles of the Fowler's toad (*Bufo woodhousii fowleri*), and determined the 96-hr LC_{50} to be 14 mg/L. The percent purity of the test material is unknown. It appears from these data that the toxicity of molinate to amphibians is comparable to the toxicity to fish.

ii. Freshwater Fish, Chronic

A freshwater fish early life-stage test using the TGAI is required for molinate because the end-use product may be applied directly to water, and the following conditions are met: (1) water monitoring data show that the pesticide is continuously presence in water over several days, (2) some aquatic acute LC_{50} and EC_{50} values are less than 1 mg/L, and (3) the EEC in water is equal to or greater than 0.01 of acute LC_{50} and EC_{50} values. Furthermore, a supplemental study with the mysid (MRID 43976801) indicates that molinate is toxic to early life-stages of aquatic organisms at concentrations considerably lower than toxic to later life-stages (MRID 43976801).

Early Life-Stage Toxicity to Freshwater Fish under Flow-through Conditions

Species, Study Duration	% ai	NOAEL (ppb)	LOAEL (ppb)	MATC[1] (ppb)	Endpoints Affected	MRID No. Author/Year	Study Classification
Rainbow trout *(Oncorhynchus mykiss)*, 60 days	99	390	830	570	Survival	40657801 McAllister 1987	Supplemental[2]

[1] MATC is defined as the geometric mean of the NOAEL and LOAEL.
[2] This study does not fulfill the guideline requirements because the dilution water had excessive hardness and pH, the time to hatch was not measured, the raw data were not provided, and incomplete information was provided on test methodology.

Based on supplemental study, survival of early life stages of freshwater fish will begin to be affected at molinate concentrations between 390 and 830 μg ai/L. It should be noted that this study does not assess the effects of molinate on the reproduction of fish. Although the test guideline (72-4) has not been fulfilled, a new fish early life-stage study is not required because information on chronic toxicity will be provided by the required fish life-cycle study (see below).

A freshwater fish life-cycle test using the TGAI is required for molinate because the end-use product is intended to be applied directly to water and both of the following triggers are met: (1) the EEC is equal to or greater than one-tenth of the NOAEL in the fish early life-stage and the invertebrate life-cycle test, and (2) studies of other organisms indicate the reproductive physiology of fish may be affected. The reproductive toxicity of molinate has been clearly demonstrated in mammalian studies, and this toxicity also may be expressed in other vertebrates, including fish. The preferred test species is fathead minnow. The test guideline (72-5) is not fulfilled.

iii. Freshwater Invertebrates, Acute

A freshwater aquatic invertebrate toxicity test using the TGAI is required to establish the toxicity of molinate to aquatic invertebrates. The preferred test species is *Daphnia magna*. Results of this test are tabulated below.

Acute Toxicity to Freshwater Invertebrates

Species, Study Type	% ai	EC_{50} (ppm ai)	Toxicity Category	MRID No. Author/Year	Study Classification
		Technical			
Waterflea *(Daphnia magna)*, static	"Technical"	*48-hr:* 19.4 (15.2-25.5)	Slightly toxic	Acc. # 246020 Vilkas and Hutchinson, 1977	Core
Waterflea *(Daphnia magna)*	Technical	*26-hr:* 0.70 (0.46-1.05)	Highly toxic	MRID 05001465 Crosby and Tucker, 1966	Supplemental[1]
Amphipod[2] (*Gammarus lacustris*)	98.6	*48-hr:* 7.6 (6.1-9.5) *96 hr*: 4.5 (3.5-5.8)	Moderately toxic	MRID 05009242 & 40098001 Mayer and Ellersieck, 1986	Supplemental
Stonefly[2] (*Pteronarcys spp.*)	98.6	*96 hr*: 0.34 (0.24-0.47)	Highly toxic	MRID 40098001 Mayer and Ellersieck, 1986	Supplemental
Moina australiensis (an Australian cladoceran), static	Technical	*48 hr:* 2.40 (1.42-4.18) *8-day:* 0.30 (0.16-0.57)	Moderately toxic Highly toxic	Julli and Krassoi, 1995	Supplemental (Open literature)
		Formulated Product			
Waterflea *(Daphnia magna)*, static	91.2 (Ordram 8E)	4.7 (4.3-5.3) (4.3 ppm ai)	Moderately toxic	MRID 41613605 Farrelly and Hamer, 1989	Core

[1] Study is supplemental because the exposure period was only 26 hours.
[2] Study was conducted with mature organisms.

The majority of the studies classify molinate as moderately to highly toxic to freshwater invertebrates on an acute basis, although one older study would classify it as slightly toxic. The stonefly EC_{50} of 0.34 mg ai/L will be used for the risk assessment to freshwater invertebrates. An 8-day study with *Moina australiensis* found an EC_{50} value that was approximately an order of

magnitude less than that found in the 48-hr toxicity study. This indicates that subacute exposure of molinate is more toxic to freshwater invertebrates than is acute exposure. A similar phenomenon has been observed in fish. The guideline (72-2) is fulfilled (MRID 41613605, Acc. No. 246020).

iv. Freshwater Invertebrate, Chronic

A freshwater invertebrate life-cycle test using the TGAI is required for molinate because the end-use product may be applied directly to water, and the following conditions are met: (1) water monitoring data show that the pesticide is continuously presence in water over several days, (2) some aquatic acute LC_{50} and EC_{50} values are less than 1 mg/L, and (3) the EEC in water is equal to or greater than 0.01 some of the acute LC_{50} and EC_{50} values. Furthermore, a supplemental study with the mysid (MRID 43976801) indicates that molinate is toxic to early life-stages of aquatic organisms at concentrations considerably lower than toxic to later life-stages (MRID 43976801). The preferred test species is the waterflea.

Life-Cycle Toxicity to Freshwater Invertebrates

Species, test type	% ai	NOAEL (ppm)	LOAEL (ppm)	MATC[1] (ppm)	Endpoints Affected	MRID No. Author/Year	Study Classification
Waterflea *(Daphnia magna)*	97.5	0.38	0.90	0.59	Reproduction and growth (length)	40657802 Forbis 1987	Core[2]
Moina australiensis (an Australian cladoceran), static	Tech	0.11 (8-day)	0.29 (8-day)	0.18 (8-day)	Reproduction	Julli and Krassoi, 1995	Supplemental (Open literature)

[1] Defined as the geometric mean of the NOAEL and LOAEL.
[2] Upgraded from supplemental to core (see EPA memo for bar code D259942)

The risk assessment will be based on core data, which shows that molinate can impair the growth and reproduction of freshwater invertebrates at concentrations of 0.90 mg/L or greater. The NOEL was established at 0.38 ppm. Supplemental data for *Moina australiensis* indicate that some freshwater crustaceans are more sensitive to molinate than is the standard test specis, *Daphnia magna*. The test guideline for an aquatic invertebrate life cycle study (GLN 72-4b) has been fulfilled (MRID 40657802).

c. Toxicity to Estuarine and Marine Animals

i. Estuarine and Marine Fish and Invertebrates, Acute

Acute toxicity testing with estuarine/marine fish and invertebrates is required because the use of molinate on rice in coastal areas is expected to result in the active ingredient reaching this environment. The preferred test species are the sheepshead minnow, the mysid and the eastern oyster. Results of these tests are tabulated below.

Acute Toxicity to Estuarine/Marine Fish and Invertebrates

Species, Type of Study	% ai.	96-hour EC50 or LC50 (ppm ai)	Toxicity Category	MRID No. Author/Year	Study Classification
Sheepshead minnow *(Cyprinodon variegatus)*, Flow-through	96.8	13-24[a]	Slightly toxic	MRID 43337604 Kent *et al.*, 1994	Supplemental[b]
Sheepshead minnow *(Cyprinodon variegatus)*, Static	98.8	13 (7.8-22)	Slightly toxic	Acc. # 258924 Ward, 1984	Supplemental[c]
Eastern oyster *(Crassostrea virginica)*, Static embryo-larvae	96.8	27.1 (25.0-29.4)	Slightly toxic	MRID 43337601 Kent *et al.*, 1994	Core
Mysid *(Americamysis bahia)* static	96.8	2.5 (1.8-3.4)	Moderately toxic	MRID 43337605 Kent *et al.*, 1994	Core
Mysid *(Americamysis bahia)* static	98.8	0.76 (0.13-1.00)	Highly toxic	Acc. # 258924	Core

[a] Observed mortality was 0% at 13 ppm and 70% at 24 ppm.
[b] The LC_{50} could not be established because mortality occurred at only one test level.
[c] This study does not fulfill the guideline requirements because insolubility was observed at the two highest test levels, fish were larger than recommended, and temperature was not properly controlled.

Since the LC_{50} for the mysid is 0.1 and 1.0 ppm, molinate is classified as highly toxic to estuarine/marine crustaceans on an acute basis. Molinate is only slightly toxic to estuarine/marine fish and immature mollusks. The guidelines for shrimp and mollusks (72-3b and 72-3c) are fulfilled (MRIDs 43739801 and 43603305, respectively). Although the guideline for estuarine/marine fish (72-3a) is not fulfilled, but supplemental data (MRID 43337604, Acc. No. 258924) and core data for the toxicity of Ordram 8E (MRID 416136-07) together provide adequate information on the acute toxicity of molinate.

Results of toxicity testing of formulated products with marine/estuarine species are given in the table below. The results indicate that the formulated products show similar toxicity to the active ingredient for fish and crustaceans, but appear to have somewhat greater toxicity to mollusks.

Acute Toxicity of Formulated Products to Estuarine/Marine Fish and Invertebrates

Species, Type of Study	Formulation	96-hour EC50 or LC50 (ppm formulation)	Toxicity Category	MRID No. Author/Year	Study Classification
Sheepshead minnow *(Cyprinodon variegatus)*, Static	Arrosolo 3-3E[1]	17 (9.4-30)	Slightly toxic	MRID 41613608 Tapp *et al*., 1990	Core
Sheepshead minnow *(Cyprinodon variegatus)*, Static	Ordram 8E[2]	12 (11-34)	Slightly toxic	MRID 416136-07 Tapp *et al*., 1990	Core
Eastern oyster *(Crassostrea virginica)*, Flow-through shell deposition	Arrosolo 3-3E[1]	4.5 (4.2-4.8)	Moderately toxic	MRID 41705302	Supplemental
Eastern oyster *(Crassostrea virginica)*, Flow-through shell deposition	Ordram-8E[2]	5.3 (4.7-6.2)	Moderately toxic	MRID 41705301	Supplemental
Mysid (*Americamysis bahia*), static	Arrosolo 3-3E[1]	7.6 (5.7-10.0)	Moderately toxic	MRID 41613610 Williams *et al.*, 1990	Core
Mysid (*Americamysis bahia*), static	Ordram-8E[2]	3.4 (2.6-4.4)	Moderately toxic	MRID 41613609 Williams *et al.*, 1990	Core

[1] Arrosolo 3-3E contains 33.5% molinate and 34% propanil.
[2] Ordram 8E contains 90.3% molinate.

ii. Estuarine and Marine Invertebrate, Chronic

An estuarine/marine invertebrate life-cycle toxicity test using the TGAI is required for molinate because the end-use product may be applied directly to the estuarine/marine environment or expected to be transported to this environment from the intended use site, and any of the following conditions are met: (1) the pesticide is intended for use such that its presence in water is likely to be continuous or recurrent regardless of toxicity, (2) any aquatic acute LC50 or EC50 is less than 1 mg/l, (3) the EEC in water is equal to or greater than 0.01 of any acute LC50 or EC50 value, or, (4) the actual or estimated environmental concentration in water resulting from use is less than 0.01 of any acute LC50 or EC50 value and any of the following conditions exist: studies of other organisms indicate the reproductive physiology of fish and/or invertebrates may be affected, physicochemical properties indicate cumulative effects, or the pesticide is persistent in water (e.g., half-life greater than 4 days). The preferred test species is the mysid. Results of this test are tabulated below.

Chronic Toxicity to Estuarine/Marine Invertebrates

Species, Type of Study	% ai	28-day NOAEL (ppb ai)	28-day LOAEL (ppb ai)	MATC[1] (ppm ai)	Most Sensitive Endpoints	MRID No. Author/Year	Study Classification
Mysid (*Neomysis mercedis*), Flow-through early life-stage test	tech	25.6	45.2	34.0	Larvae survival	MRID 43976801 Bailey 1993	Supplemental[2]

[1] Defined as the geometric mean of the NOAEL and LOAEL.
[2] This study is supplemental due to guideline deviations in hardness and pH, no time-to-hatch data, no raw data submitted, and incomplete information of test methodology.

Based on measured concentrations, molinate inhibited the growth of mysids at concentrations of 6.15 μg/L and greater. The NOAEL for growth was 3.09 μg/L. Reproduction of mysid was impaired at a concentration of 45.4 μg/L. The test guideline (72-4) is not fulfilled.

d. Toxicity to Plants

i. Terrestrial Plants

Terrestrial plant testing is required for molinate because it is an herbicide with non-residential terrestrial use patterns, and it may be applied aerially, is volatile (vapor pressure = 5.3×10^{-3} mg Hg/Torr at 25°C), and may pose hazards to endangered or threatened plant species. The required testing consists of seedling emergence and vegetative vigor tests with ten crop species. Six of the species must be dicotyledonous and represent at least four families. One of these species must be soybean (*Glycine max*) and a second must be a root crop. The remaining four species must be monocotyledonous and represent at least two families. One of these species must be corn (*Zea mays*). Tier I tests (GLN 122-1) may be conducted to measure the response of plants, relative to a control, at a test level that is equal to the highest use rate (expressed as lbs ai/A) or three times the EEC for nontarget areas. Tier II tests (GLN 123-1) are required for any test species that shows a reduction in response equal to or greater than 25% in the Tier I tests.

Tier II tests measure the response of plants, relative to a control, and five or more test concentrations. A tier II phytotoxicity study with Ordram 8E, a TEP containing 91.4% molinate, has been submitted to the Agency (MRID 41613611). The seedling emergence portion of this test was classified as invalid and cannot be used to assess risk. The results of the vegetative vigor portion of this study are tabulated below.

Effects of Ordram 8-E (91.4% Molinate) on the Vegetative Vigor of Nontarget Terrestrial Plants (Tier II), Based on Farmer and Canning, 1990 (MRID 41613611).

Species	EC_{25} (lbs ai/A)	EC_{10} (lb ai/A)[a]	Most Sensitive Endpoint Affected	Study Classification
Corn (monocot)	>4.0	1.4 (0.74-2.7)	Visual assessment	Supplemental[c]
Winter wheat (monocot)	>4.0	>4.0	Visual assessment	Supplemental[c]
Wild oat (monocot)	>4.0	2.9 (1.0-45)	Visual assessment	Supplemental[c]
Purple nutsedge (monocot)	>4.0	>4.0	Visual assessment	Supplemental[c]
Sugar beet (dicot, root crop)	>4.0	1.3 (0.63-2.6)	Visual assessment	Supplemental[c]
Soy bean (dicot)	0.22 (0.15-0.29)	0.05 (0.03-0.78)	Visual assessment	Supplemental[c]
Oilseed rape (dicot)	2.5 (1.6-4.9)[b]	0.32 (0.15-0.54)[b]	Visual assessment	Supplemental[c]
Teaweed (dicot)	1.3 (1.0-1.6)	0.62 (0.44-0.81)	Visual assessment	Supplemental[c]
Velvetleaf (dicot)	0.42 (0.31-0.53)	0.13 (0.08-0.19)	Visual assessment	Supplemental[c]
White mustard (dicot)	0.66 (0.41-1.0)	0.06 (0.02-0.12)	Visual assessment	Supplemental[c]

[a] EC_{10} values are reported in lieu of NOAEL values, which were not determined for the visual damage assessment. For plant dry weights, the study report gave NOAEL values, but not EC_x values. For every species tested, the NOAEL value for plant dry weights was greater than the EC_{10} for the visual damage assessment.
[b] The EC_{25} and EC_{10} values reported for oilseed rape are for 14 days after treatment because heat damage to plants prevented data from being collected for 28 days after treatment.
[c] This study is supplemental because EC values were not calculated for percent emergence or dry weight, visual assessments were not conducted in control plants, and the progression of application rates was 4-fold rather than 2-fold.

For Tier II vegetative vigor soybean is the most sensitive species (based on supplemental data). Monocot species do not appear to be sensitive to vegetative vigor effects of molinate. The test guidelines (123-1a and 123-1b) are not fulfilled.

ii. Aquatic Plants

Terrestrial Tier II studies are required for all low dose herbicides (those with the maximum use rate of 0.5 lbs ai/A or less) and any pesticide showing a negative response equal to or greater than 50% in Tier I tests. The following species should be tested at Tier II: *Pseudokirchneria subcapitata* (formerly *Selanastrum capricornutum*), *Lemna gibba*, *Skeletonema costatum*, *Anabaena flos-aquae*, and a freshwater diatom.

Results of Tier II toxicity testing on the technical/TEN material are tabulated below.

Toxicity to Aquatic Plants (Tier II)

Species	% ai	EC_{50} (ppm)	NOAEL (ppm)	MRID No. Author/Year	Study Classification
			Vascular Plants		
Duckweed *Lemna gibba*	99	3.30	0.84	MRID 41702702 Thompson *et al.*, 1990	Core
			Nonvascular Plants		
Green algae *Pseudokirchneria subcapitata*	99	0.22 (4-day)	0.17 (4-day)	MRID 41613612 Smyth *et al.*, 1990	Core
Marine diatom *Skeletonema costatum*	99	4.3 (4-day)	0.94 (4-day)	MRID 41613613 Smyth *et al.*, 1990	Core
Freshwater diatom *Navicula pelliculosa*		10 (4-day)	4.5 (4-day)	41702703	Core
Blue-green algae *Anabaena flos-aquae*		9.48	0.95	41702701	Supplemental

The Tier II results indicate that *Pseudokirchneria subcapitata* is the most sensitive aquatic plant, with an EC_{50} of 0.22 ppm. The guideline (123-2) is fulfilled for duckweed, green algae, marine diatoms, and freshwater diatoms (MRID 41702702, 41613613, 41613613, and 41702703), but not for bluegreen algae.

e. Fish and Wildlife Mortality Incidents

Finlayson and Faggella (1986) investigated the cause of large fish kills that occurred in the Colusa Basin Drain in north-central California, an agricultural drain that runs through an intensive rice-growing area. They report that an estimated 7,000 to 30,000 carp died annually in this drain between 1981 and 1983. No fish kills were observed in 1984 to 1987. They determined the 28-day LOEL and LC_{50} for carp to be 0.13 and 0.21 ppm, respectively. Water monitoring of the Colusa Basin Drain showed that peak residue values in the years 1981 through 1983 exceeded the carp LOEL and approached or exceeded the carp LC_{50}, whereas peak values during 1984 through 1986 remained below the carp LOEL and LC_{50}. Significantly, primarily carp were killed in this drain, while other species present such as bass and catfish were not affected. Laboratory studies have shown that carp are particularly sensitive to the anemic effects of molinate, whereas other species, such as the channel catfish (Finlayson and Faggella, 1986) and bluegill sunfish (Miller, 1984) are relatively insensitive. Furthermore, Finlayson and Faggella (1986) showed that molinate exposure significantly reduces hemoglobin and hematocrit levels in carp, and found that carp collected from the Colusa Basin Drain in 1983 had reduced hemoglobin and hematocrit on June 15, following a period of high molinate exposure. Thus, while there is no evidence from tissue analysis, circumstantial evidence suggest that molinate was likely the primary causal factor of the large fish kills in the Colusa Basin Drain. It should be noted that continued residue monitoring of this drain has shown that peak residues were reduced substantially following the imposition of

mandatory water-holding periods for rice farmers beginning in 1990.

The Environmental Incident Information System contains two incidents of fish kills that could be attributed to molinate. In one incident in 1978, thousands of dead carp were observed in the a toe drain area on the Yolo Bypass in Yolo County, California (Incident # B0000-215-06). Analysis of water samples detected molinate at a concentration of 60 ppb. Fish were too decomposed for tissues to be analyzed. Although molinate cannot be confirmed as the causal factor, the similarities of this incident to the kills of carp mentioned above suggest that it could have been caused by molinate toxicity.

A second incident possibly attributed to molinate occurred in Bayou Macon in West Carroll Parish, Louisiana, in 1991 (Incident # I000109T). This incident involved an unknown number of dead fish of "multiple species". It occurred in a bayou that is lined on both sides by cotton and rice fields. Water samples revealed molinate concentrations of 15-20 ppb. Although this concentration is not thought to be enough to be lethal to fish, the condition of the fish indicated that they were killed several days prior to the incident being reported, and molinate residues could have been higher at that time. No analysis of fish tissue was conducted since the fish were too decomposed. There is too little evidence to conclude that this fish kill was or was not caused by molinate.